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Changes in the central ANF-system of renovascular hypertensive rats

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Changes in the central ANF-system of renovascular hypertensive rats. The central atrial natriuretic peptides (ANF)-system was investigated in volume-dependent one-kidney, one-clip (1K1C) and renin-dependent two-kidney, one-clip (2K1C) renovascular hypertensive rats by radioimmunological measurement of ANF concentration in 18 selected brain areas. Significant changes were found in nine brain areas of 1K1C and in eight brain areas of 2K1C hypertensive rats. Except unidirectional changes in the organum vasculosum laminae terminalis and the supraoptic nucleus, ANF concentration was changed in the opposite direction in all other brain areas, with an activation of the central ANF system in 1K1C and an inhibition in 2K1C hypertension. The localization of the alterations (circumventricular organs, anteroventral third ventricle region, hypothalamo hypophyseal system, brain stem) implies major differences in the central regulation of blood pressure and electrolyte and fluid homeostasis between these two models. The activation of the central ANF system in 1K1C hypertension may be a compensatory mechanism to prevent further increments in blood pressure and plasma volume. In contrast, the depression of the central ANF system in 2K1C hypertension may promote the elevation of the blood pressure.

Since Goldblatt et al [1] demonstrated the development of arterial hypertension following renal ischemia, the pathogenesis of renovascular hypertension has been intensively investigated. However, the mechanism of the hypertension in renal artery stenosis is still not completely understood. Clipping of one renal artery regularly induces hypertension, but there are differences between the two-kidney, one-clip (2K1C) model, in which the contralateral kidney is untouched, and the one-kidney, one-clip (1K1C) model, in which the contralateral kidney is removed. The 2K1C model seems to be dependent on the renin-angiotensin system, while the 1K1C model is suggested to be volume expanded [2–5].

There is an increasing number of indications that the atrial natriuretic factor (ANF), a peptide hormone that is intimately involved in the regulation of renal and cardiovascular homeostasis [6–8], may play an important role is the pathomechanism of renovascular hypertension. In 1K1C rats plasma ANF levels are positively correlated with blood pressure and negatively correlated with ANF concentration in the left atrium [9]. Additional infusion of synthetic ANF results in a reduction of blood pressure and increased diuresis and natriuresis in 1K1C rats [10]. Unclipping of the remaining kidney is accompanied by a reversal of hypertension, which might be partially due to the still higher plasma ANF levels [9]. Based on these findings, it was suggested that the increased plasma ANF level in 1K1C hypertension reflects a compensatory mechanism preventing further increments in blood pressure and plasma volume. In 2K1C rats plasma ANF level is unchanged, but chronic administration of synthetic ANF also decreases blood pressure in renin-dependent 2K1C hypertension [11]. The hypotensive effect of ANF in this model may be related to the inhibition of renin release and the consequent decrease of plasma angiotensin II and aldosterone levels [12].

However, the mechanisms underlying the shifting pattern of hemodynamic and humoral events in the various stages and different forms of renal hypertension may not only be localized in the periphery but may be combined with regulatory mechanisms in the central nervous system. Thus, it was shown that 1K1C hypertension is associated with an increased activity of the sympathetic nervous system [13,14], and that ablation of the anteroventral third ventricle (AV3V) region prevents the development of 2K1C hypertension [15,16]. Since the central ANF system is known to be important for the regulation of electrolyte and fluid homeostasis and blood pressure [17–21], it may also be involved in the pathophysiology of renovascular hypertension. To elucidate this question, we have investigated the central ANF system in the early maintenance phase of 1K1C and 2K1C renovascular hypertension, when differences in the pathological disorders could be expected. For this purpose, the concentration of ANF was measured radioimmunologically in 18 selected brain areas of 1K1C and 2K1C hypertensive rats and compared with the respective controls. Previous studies have shown that the concentration of ANF in specific brain areas is a sensitive parameter for alterations in the electrolyte and fluid homeostasis and blood pressure [22–24].

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Methods

Protocol

Renovascular hypertension was produced in male Sprague-Dawley rats (Charles River Wega GmbH, Sulzfeld, FRG; weight 80 to 100 g; age 35 to 40 days) under sodium pentobarbital anesthesia (60 mg/kg body wt i.p.) by partial constriction of the left renal artery with a silver clamp of 0.20 mm internal diameter. In the 1K1C hypertension group the contralateral kidney was removed simultaneously, whereas it was left untouched in the 2K1C hypertension group. Animals subjected to sham operation, in which a silver clamp was temporarily placed around the left renal artery and then taken away and the right kidney was removed (1K1C control group) or left untouched (2K1C control group), served as normotensive controls. The animals were housed at a constant room temperature of 22° ± 1° C and a humidity of 60% ± 4%, with illumination from 6.00 a.m. to 6.00 p.m. Systolic blood pressure was measured indirectly once a week by means of the tail cuff technique. The criterion for classifying renovascular hypertensive and sham operated animals were as follows: clipped animals having a systolic blood pressure higher than or equal to 160 mm Hg (about 70% of all clipped animals) were classified as renovascular hypertensive rats; all animals selected for the 2K1C hypertension group had to have a four fold increased plasma renin activity; sham operated animals having a systolic blood pressure less than or equal to 120 mm Hg (about 90% of all sham operated animals) were classified as normotensive.

Determination of blood parameters

The blood parameters were determined separately in 10 animals per experimental group. The animals were chosen randomly and moved to a quiet room. One at a time they were transferred to an adjacent room.

For determination of hematocrit, plasma osmolality, serum sodium, potassium and chloride concentration blood was collected by puncture of the aorta under sodium pentobarbital anesthesia (60 mg/kg body wt i.p.). Hematocrit was determined by an automatic analyzer (ELT 8, Fa. Moltronic, Heidelberg, FRG), plasma osmolality by freezing point depression (Osmomat, Fa. Gonotec, Berlin, FRG), serum sodium and potassium by flame photometry (AFM 2, Eppendorf Co., Hamburg, FRG) and chloride by mercurymetry (Chlorimeter, Eppendorf Co., Hamburg, FRG).

For determination of plasma renin activity (PRA) and angiotensin II (Ang II) concentration rats were rapidly anesthetized with ether, a laparatomy performed, and blood collected from the abdominal aorta. The interval between the beginning of the exposure to ether and the end of the blood withdrawal never exceeded 150 seconds. Blood was collected into prechilled (4°C) polypropylene tubes, which contained 5% of a solution of 125 mM EDTA for determination of PRA or 5% of a solution of 125 mM EDTA and 26 mM orthophenantroline for determination of Ang II concentration. After centrifugation (6000 g, 10 min, at 4°C) plasma was stored at −20°C until assay. PRA was determined by radioimmunological measurement of generated angiotensin I [25] and Ang II was measured by direct radioimmunoassay in unextracted plasma [26].

Plasma aldosterone concentration was measured in heparinized, unextracted plasma by a commercial aldosterone radioimmunoassay (Biermann Diagnostica, Bad Nauheim, FRG).

Plasma arginine vasopressin concentration was measured after extraction from plasma by radioimmunoassay as described elsewhere [27].

For determination of plasma norepinephrine concentration blood was collected during the first 3 seconds after rapid decapitation into prechilled (4°C) polypropylene tubes containing EDTA. The measurement was performed by high-pressure liquid chromatography using a Beckman 114 M pump and a Chromatopak C-18 column. The samples were eluted in an isocratic system with a 0.06 M phosphate buffer at a flow rate of 0.5 ml/min. The detection was carried out electrochemically at + 650 mV towards an Ag/AgCl electrode [28].

For determination of plasma ANF concentration, blood was taken from the aorta, collected in polypropylene tubes containing 500 KIU aprotinin (Trasylol^R, Bayer AG, Leverkusen, FRG), and immediately centrifuged (2.000 g, 10 min, at 4°C). The plasma was stored at −70°C until assay. ANF was extracted from plasma according to the method of Larose et al [29] modified in some details. Plasma sample (1 ml) acidified to pH 3 to 4 with 2 N HCL was applied to a Sep Pak C18 reverse-phase octadodecyl silane cartridge column (Water Associates, Milford, Massachusetts, USA) that had been activated previously with 100% methanol (10 ml) followed by 5% acetic acid (10 ml). After washing with trifluoracetic acid (TFA, 3 × 5 ml), the peptide was eluted with a mixture of 60% acetonitrile in 0.1% TFA (2 ml). The eluates were evaporated to dryness with nitrogen, and the residue was reconstituted with 1 ml of RIA buffer (50 mM potassium phosphate buffer, pH 7,4, containing 0.1% BSA, 0.02 NaN_3, 50 mM NaCl, 0.1% Triton X-100, 50 μM phenylmethylsulfonyl fluoride and 10 μM aprotinin) for ANF determination by radioimmunoassay.

Tissue dissection and extraction of brain ANF

The animals were decapitated between 8.00 and 10.00 a.m., brains were quickly removed and frozen on dry ice. Serial coronal sections with 300 μm thickness were cut in a cryostat on −10°C and 18 brain regions (Table 2) were removed by the micropunch technique [30]. The brain regions represented those functional systems which were mainly involved in the regulation of electrolyte and fluid homeostasis and blood pressure. Tissue pellets of identical brain nuclei of four animals were pooled, and six assay sets per group were formed (4 × 24 rats). The samples were collected in 1.5 ml conical Eppendorf tubes containing 60 μl 0.1 N HCL and put in a boiling water bath for 10 minutes. After addition of 100 μl RIA-buffer the samples were homogenized and 20 μl aliquots of the homogenates were removed for protein determination according to Lowry et al [31]. The remaining sample was centrifuged at 2000 g for 15 minutes at 4°C and 100 μl of the supernatant fluid were used for ANF determination by radioimmunoassay.

Measurement of plasma ANF and brain ANF concentration

For determination of plasma ANF and brain ANF concentration a commercial radioimmunoassay kit was used (Peninsula Labs., INC, Belmont, California, USA; RIK 8798). The rabbit antihuman ANF (99-126) antiserum of this kit cross reacts with the different atrial natriuretic peptides as follows: 100% with

Table 1. Characteristics of 1K1C and 2K1C renovascular hypertensive rats and their control rats (mean ± SEM)

	1K1C control	1K1C	2K1C control	2K1C
No.	10	10	10	10
Body weight *g*	262.0 ± 8.5	246.5 ± 9.5	266.0 ± 8.5	232.5 ± 7.0
Systolic blood pressure *mm Hg*	112.5 ± 3.5	178.0 ± 4.5[c]	116.5 ± 3.5	175.0 ± 5.0[c]
Water intake *% of body wt*	15.5 ± 1.5	10.8 ± 2.6[a]	15.0 ± 0.5	23.0 ± 1.2[a]
Urine volume *% of body wt*	7.3 ± 0.9	5.3 ± 0.6	8.0 ± 0.4	11.0 ± 1.3[a]
Hematocrit *%*	41.7 ± 0.5	38.1 ± 0.4[a]	42.1 ± 0.4	46.1 ± 0.4[a]
Plasma sodium concentration *mmol/liter*	147.7 ± 1.0	141.2 ± 1.6[b]	150.8 ± 0.6	148.1 ± 1.4
Plasma potassium conc. *mmol/liter*	4.4 ± 0.1	4.5 ± 0.2	4.5 ± 0.2	4.4 ± 0.2
Plasma chloride conc. *mmol/liter*	99.5 ± 1.3	98.0 ± 0.4	103.7 ± 0.7	101.7 ± 1.5
Plasma osmolality *mOsm/kg water*	315.4 ± 1.4	302.4 ± 2.0[b]	318.6 ± 2.4	316.2 ± 1.9
Plasma renin activity *ng Ang I/ml/hr*	1.5 ± 0.2	1.4 ± 0.2	1.9 ± 0.4	9.8 ± 0.6[c]
Plasma angiotensin II conc. *fmol/ml*	152.3 ± 23.1	147.1 ± 16.2	150.5 ± 21.0	956.5 ± 93.5[c]
Plasma aldosterone conc. *fmol/ml*	81.0 ± 19.7	67.2 ± 16.2	94.5 ± 20.2	486.0 ± 81.0[b]
Plasma norepinephrine conc. *pmol/liter*	1.0 ± 0.3	3.5 ± 0.4[b]	1.3 ± 0.2	1.4 ± 0.3
Plasma arginine vasopressin conc. *pg/ml*	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	2.6 ± 0.4[b]
Plasma ANF concentration *pg/ml*	7.2 ± 0.8	29.6 ± 2.5[c]	8.0 ± 1.3	12.6 ± 1.8

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, significant difference from the renovascular hypertensive rats to their respective control rats

human ANF (99-126), rat ANF (99-126) and rat ANF (102-126), 90% with rat ANF (101-126), 88% with rat ANF (103-126), 57% with ANF (108-126), 50% with rat ANF (111-126), 27% with rat ANF (103-125), 10% with rat ANF (102-125) and 3% with rat ANF (103-123). The ANF antiserum does not cross react with somatostatin, oxytocin, arginine[8]-vasopressin and brain natriuretic polypeptide porcine (BNP). In conclusion, the antiserum recognizes nearly completely the active forms of ANF as well in the plasma (rat ANF (99-126)) [7,32] as in the brain (rat ANF (102-126) and rat ANF (103-126)) [33,34].

The reconstituted plasma extracts (100 µl), respectively, the brain extracts (100 µl) were incubated with the same volume of the antiserum. Synthetic human ANF (99-126) ranging from 1 to 128 pg/tube was incubated as standard. After incubation for 24 hours at 4°C, 100 µl of the ANF tracer ^{125}J-human ANF (99-126) was added. After again 24 hours incubation at 4°C, goat anti-rabbit IgG serum and normal rabbit serum (100 µl, dissolved in RIA buffer) were added and the mixture was incubated at room temperature for two hours. Then 500 µl RIA buffer was added, the contents were vortex mixed and then centrifuged (2000 g, 20 min, at 20°C). After centrifugation the supernatants were aspirated and the precipitants were counted in a gamma counter. The sensitivity of the assay was 1.5 pg/tube. The recovery of added ANF standard was 78.7 ± 3.3% (mean ± SEM, $N = 6$) and the intraassay and interassay variations were 9% and 14%, respectively. Serial dilutions of plasma and brain extracts showed parallelism to the synthetic ANF standards.

Statistical analysis

The results are expressed as mean ± SEM. Statistical significance was tested by the Student's *t*-test. Differences at the 95% level were considered significant.

Results

Animal characteristics

Data are summarized in Table 1. Five weeks after surgery the mean blood pressure was 178 mm Hg in 1K1C rats and 175 mm Hg in 2K1C rats; the development of blood pressure is shown in Figure 1. In 1K1C hypertensive rats there was a significant decrease in water intake, hematocrit, plasma sodium concentration and plasma osmolality; plasma ANF and plasma norepinephrine concentrations were significantly increased. In contrast, 2K1C-hypertensive rats showed a significant increase in water intake, urine volume and hematocrit. The measurement of different components of the renin-angiotensin-aldosterone system revealed an expressed stimulation of this system with an about fivefold increase of plasma renin activity, plasma angiotensin II and plasma aldosterone concentration. Furthermore, plasma arginine vasopressin concentration was significantly increased.

ANF concentration in specific brain areas

The comparison of ANF concentrations in renovascular hypertensive rats with their normotensive control rats showed significant differences in nine brain areas of 1K1C hypertensive rats and in eight brain areas of 2K1C hypertensive rats (Table 2). In 1K1C hypertensive rats ANF concentration was significantly decreased only in the organum vasculosum laminae terminalis (OVLT) (28% of control). In all other brain areas with changes ANF concentration was significantly increased. Very pronounced elevations were found in the subfornical organ (211%), the supraoptic (244%) and paraventricular (172%) nuclei, the arcuate nucleus (245%), the median eminence (231%), the periventricular preoptic nucleus (169%) and the locus coeruleus (206%). The increase in the dorsal raphe nucleus (134%) was just moderate. In contrast, in 2K1C hypertensive rats ANF concentrations were increased only in the supraoptic nucleus (202%) and the medial amygdaloid nucleus (179%), whereas a significant decrease was found in the cerebral cortex (52%), the OVLT (36%), the periventricular preoptic nucleus (33%), the nucleus interstitialis striae terminalis (NIST) (59%), the median eminence (60%) and the medial forebrain bundle (69%).

Discussion

Present data confirm that there are differences in the pathogenesis of 1K1C and 2K1C hypertension. In 1K1C rats we have

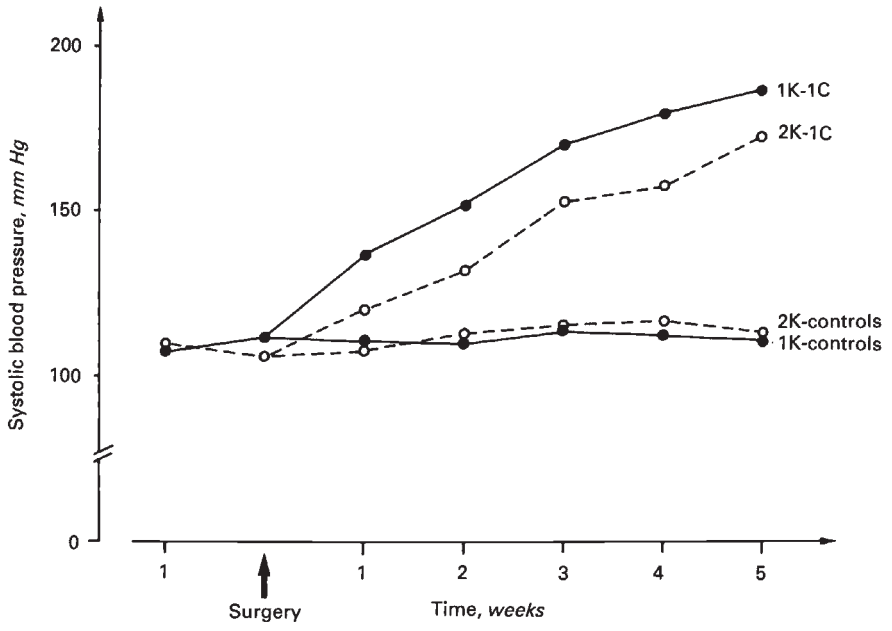

Fig. 1. *Development of blood pressure in 1K1C and 2K1C renovascular hypertensive rats and their respective control rats.* Symbols are: (●) 1K1C on top, 1 kidney controls on bottom line; (○) 2K1C on top, 2 kidney controls on bottom dashed line.

Table 2. Concentrations of ANF in selected brain areas of 1K1C and 2K1C renal hypertensive rats and their control rats (pg ANF/mg protein; mean ± SEM)

Brain area	1K1C control	1K1C	2K1C control	2K1C
Cerebral cortex	46.2 ± 7.8	42.4 ± 6.1	42.7 ± 4.5	22.2 ± 2.1[c]
Org. vasc. lam. term.	83.0 ± 7.0	23.0 ± 1.7[c]	107.1 ± 3.7	38.7 ± 6.5[c]
Subfornical organ	73.6 ± 6.8	155.6 ± 8.7[b]	76.6 ± 15.8	86.0 ± 19.8
Periventr. preopt. nucl.	337.5 ± 16.0	570.8 ± 75.8[a]	400.6 ± 47.3	134.2 ± 18.3[c]
Medial preopt. nucl.	237.8 ± 9.4	235.0 ± 20.0	219.6 ± 35.3	173.1 ± 12.8
NIST	140.6 ± 20.9	214.8 ± 21.8	123.7 ± 13.3	73.3 ± 7.2[b]
Periventr. hypoth. nucl.	256.1 ± 50.3	182.2 ± 26.7	190.6 ± 22.6	173.6 ± 9.9
Supraoptic nucl.	44.2 ± 8.1	107.8 ± 9.0[b]	47.9 ± 5.9	96.8 ± 10.4[b]
Paraventricular nucl.	146.0 ± 13.1	251.7 ± 15.7[b]	153.9 ± 9.2	177.0 ± 19.1
Arcuate nucl.	125.8 ± 12.8	308.6 ± 42.4[c]	109.1 ± 16.3	116.5 ± 19.6
Median eminence	175.9 ± 15.3	406.0 ± 69.2[b]	152.5 ± 13.5	91.0 ± 7.7[b]
Perifornical nucl.	99.5 ± 16.6	106.1 ± 23.3	77.9 ± 7.2	95.4 ± 9.6
Median forebrain bundle	62.6 ± 6.6	49.0 ± 6.2	61.4 ± 4.0	42.4 ± 4.5[a]
Medial amygd. nucl.	42.3 ± 4.0	63.7 ± 12.1	31.3 ± 4.3	56.2 ± 9.4[a]
Dorsal raphe nucl.	90.6 ± 12.2	121.2 ± 8.7[a]	104.3 ± 14.9	115.0 ± 24.7
Tegmentum pontis	118.6 ± 21.6	70.3 ± 11.6	95.4 ± 10.0	83.9 ± 6.3
Locus coeruleus	62.9 ± 9.4	129.8 ± 14.9[b]	79.9 ± 3.8	82.8 ± 3.3
Nucl. tractus solitarii	74.3 ± 5.6	65.0 ± 8.3	54.2 ± 4.1	51.6 ± 3.8

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, significant difference from the renovascular hypertensive rats to their respective control rats

found a normal plasma renin activity but a significantly increased plasma ANF level, which might be elevated in response to the augmented plasma volume in these animals. In contrast, the hypertension in our 2K1C model seems to be clearly renin dependent, since we have found an almost fivefold increase of plasma-renin-activity, plasma angiotension II and plasma aldosterone concentration. The unchanged plasma ANF level and the significantly increased water intake tell against a relevant volume load in these animals.

The determination of ANF concentration in specific brain areas shows differential changes in 1K1C and 2K1C hypertensive rats. Except the unidirectional changes in the organum vasculosum laminae terminalis and in the supraoptic nucleus, ANF is changed in the opposite direction in all other brain areas, with an activation of the central ANF system in volume-dependent 1K1C hypertension and an inhibition in renin-dependent 2K1C hypertension. In both models significant alterations were found in the circumventricular organs, in areas of the anteroventral third ventricle (AV3V) region and in the hypothalamohypophyseal system. In 1K1C hypertensive rats ANF was furthermore elevated in brain areas which are involved in the regulation of the sympathetic nervous system.

Of special interest are the alterations in the two investigated circumventricular organs, the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT). These brain areas lack a blood brain barrier and are exposed to changes in levels of circulating peptides such as ANF and may therefore represent the brain sites of action for circulating ANF. The SFO has long been implicated in the central regulation of electrolyte and fluid homeostasis and blood pressure [35–37]. In addition to

very high numbers of ANF receptors it contains also very high concentrations of angiotensin II (Ang II) receptors and has been shown to be the main brain target site for circulating Ang II [38]. Stimulation of Ang II receptors results in increased thirst, increased vasopressin secretion and increased blood pressure [39]. Since these actions are opposed by ANF [17–20,40] a local antagonism between these two peptides was postulated to exist in the SFO. In this regard, it is conceivable that the SFO might also be involved in the pathomechanism of renovascular hypertension. The increased binding of ANF in the SFO of 1K1C hypertensive rats could be a mechanism by which volume overload is signalled to intracerebral regulatory systems. In contrast, the unchanged ANF concentration in the SFO of 2K1C rats indicates that an imbalance between ANF and A II might exist there in these rats, which could be transferred to structures inside the blood brain barrier.

The OVLT contains not only ANF receptors, but also large numbers of ANF-immunoreactive nerve terminals and cell bodies. The coexistance of ANF receptors and ANF containing terminals outside the blood brain barrier suggests the possibility of an intimate connection between the peripheral and the central peptide system in the OVLT. Topographically and functionally, the OVLT belongs to the so-called anteroventral third ventricle (AV3V) region [41], which includes additionally the preoptic and hypothalamic periventricular nuclei and a part of the medial preoptic nucleus. This region incorporates the largest accumulation of ANF containing cells in the brain [21, 34]. It is sensitive to osmotic stimuli [41] and known to integrate, at a central level, mechanisms which modulate complex homeostatic systems, such as those associated with body fluid and electrolyte balance as well as cardiovascular regulation [41,42]. The importance of this brain region for the development of renovascular [15,16], Dahl [43] and deoxycorticosterone acetate salt [15] hypertension is well known. The changes of ANF concentration in the AV3V region of our models imply a special role for ANF mediated mechanisms at this level. In the OVLT, ANF is significantly decreased in both 1K1C and 2K1C hypertensive rats. Since a similar decrease was also found in spontaneously hypertensive rats [22], we suggest that ANF is decreased there as a consequence of the raised blood pressure. However, the mechanism remains to be elucidated.

Significant changes of ANF concentration have also been found in the hypothalamic supraoptic (SON) and paraventricular nuclei (PVN), where vasopressin is produced. On the basis of neuroanatomical and electrophysiological studies [19,44–48] it was suggested that vasopressin secretion is integrated by a regulatory unit which is composed of structures in the SFO, the AV3V region, the SON and PVN and uses Ang II and ANF as antagonistic neurotransmitters. The changes of ANF concentration in this regulatory unit indicate an altered influence on vasopressin secretion in both models of renovascular hypertension. But the significantly increased ANF level in 1K1C and 2K1C rats may be caused by different mechanisms. In volume loaded 1K1C rats ANF may be elevated there to inhibit vasopressin secretion. However, the unchanged plasma vasopressin concentration indicates that vasopressin secretion may still be too high for the state of volume dependent hypertension. In renin-dependent 2K1C rats ANF may be increased there to counteract the Ang II-stimulated vasopressin secretion. Interestingly, ANF in the PVN is only increased in 1K1C rats. This may be explained by the different functional activity of the PVN and SON. While vasopressin and oxytocin secretion is dominant in the SON, the function of the PVN is more complex. Only the magnocellular subdivision of the PVN is engaged in vasopressin and oxytocin secretion, but the parvocellular subdivision contains several other neurotransmitters and neurohormones which are mainly involved in the regulation of the anterior pituitary gland [49]. Furthermore, cells in the PVN are integrated in a neural network with structures in the brain stem and the spinal cord and thereby involved in the regulation of blood pressure [50]. The increased ANF content in the PVN of 1K1C rats might therefore be related with these functions.

In this context it is of interest, that in the locus coeruleus ANF is increased only in 1K1C rats. The locus coeruleus is the quantitatively most important noradrenergic cell group of the brain and known to be involved in blood pressure regulation by modulation of the sympathetic nervous activity. It was shown that ANF can inhibit the increased sympathetic nervous activity in 1K1C hypertensive rats [51]. Therefore, we conclude that the increased ANF concentration in the locus coeruleus aims at an inhibition of the sympathetic nervous activity at this level and thereby counteracts the increased blood pressure. Interestingly, ANF is also increased in the locus coeruleus of DOCA-salt hypertensive rats; this form of hypertension is also volume dependent [38].

In conclusion, the central ANF system is differentially activated in 1K1C and 2K1C renovascular hypertension. In 1K1C hypertension, the activation of the central ANF system may be a compensatory mechanism to prevent further increments in blood pressure and plasma volume. In contrast, the depressed activity of the central ANF system in 2K1C hypertension may be a factor by which the elevation of blood pressure is promoted.

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